

THE DOMAIN FOR TRANSFER RIBONUCLEIC ACID BINDING TO THE *ESCHERICHIA COLI* RIBOSOME

M. USTAV, M. SAARMA, A. LIND and R. VILLEMS

Laboratory of Molecular Biology, Tartu University, Tartu 202400, Estonia, USSR

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1. Introduction

Although an interaction between tRNA and ribosomal proteins was strongly suspected earlier, the first experimental proof was obtained only recently [1–4]. It was shown that a set of ribosomal proteins containing both 30 S subunit and 50 S subunit proteins, interact with tRNA immobilized to Sepharose [1,2]. Also, separately from each other, a number of both 30 S and 50 S subunit proteins were found to bind to immobilized tRNA [3,4]. In this paper we show that there is cooperativity between 30 S and 50 S subunit proteins in the assembly of tRNA–protein complex. An excellent fit of the presented data with the immuno-electron microscopic mapping of proteins on the surface of the ribosome allows the localization of a domain of proteins involved in tRNA binding.

2. Experimental

All experimental procedures used in this work were as in [3,4]. The columns containing 0.3–0.5 ml RNA–gel (corresponds to 1–2 mg tRNA) were equilibrated with Tris–HCl buffer, pH 7.5, 6 mM 2-mercaptoethanol and various concentrations of MgCl₂ and potassium chloride. A sample of 70 S ribosomal proteins at a concentration of 0.2–0.5 mg/ml in a volume of 2–5 ml was passed through the column at 4°C. The

column was then washed with about 50 vol. buffer after which bound proteins were eluted by 1 M potassium chloride in 10 mM Tris–HCl buffer, pH 7.5, 5 mM EDTA and 6 mM 2-mercaptoethanol. The eluted proteins were precipitated in cold 10% trichloroacetic acid and analyzed by twodimensional urea–urea gel-electrophoresis system [5].

3. Results and discussion

There are a number of reasons for using epoxy-activated Sepharose 6B in our experiments. Firstly, this Sepharose reacts easily with adipic acid dihydrazide, thus giving a convenient way to immobilize an RNA via its oxidized 3'-end ribose. Secondly, taking together the two radicals, the 'epoxy arm' and the dihydrazide of adipic acid, we got a rather hydrophilic spacer group with total length 30 Å. Both its hydrophilicity and its length will be likely to reduce possible steric hindrances in the interaction of proteins with an immobilized RNA. Thirdly, without RNA bound to Sepharose the latter does not bind ribosomal proteins even at a low ionic strength.

It was possible to immobilize up to 20 mg tRNA/g dry gel with a yield better than 80%. Using ³²P-labelled tRNA it was shown that if kept in the neutral buffer at 4°C, less than 3% of the tRNA is released during 8 weeks.

Various control experiments were detailed [3,4]. Besides those, one more criterion for specificity became evident from the fact that proteins bound to the immobilized tRNA can be washed out from the gel with a binding buffer solution containing excess

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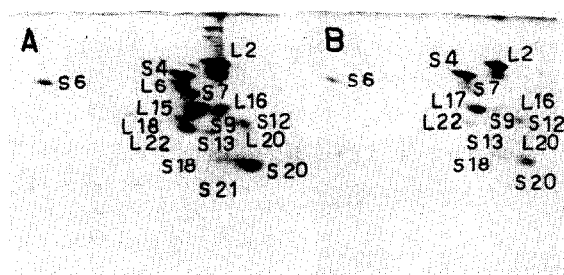


Fig.1. Identification of the *E. coli* 70 S ribosomal proteins bound to the immobilised tRNA at 4°C. 70 S ribosomal proteins, 3.8 mg in 10 ml, were passed through the Sepharose column containing 2.1 mg rat liver tRNA in 0.4 ml. (A) 70 S ribosomal proteins bound in 1 mM MgCl₂, 200 mM KCl. (B) 70 S ribosomal proteins bound in 30 mM MgCl₂, 100 mM KCl; other buffer components are indicated in the text. Bound proteins, 0.43 mg and 0.20 mg, respectively, were used for analysis. Two-dimensional polyacrylamide gel electrophoresis system was as in [5].

tRNA (data not shown). Therefore, it is indeed tRNA which interacts with ribosomal proteins.

Two-dimensional analysis of 70 S ribosomal proteins is illustrated in fig.1. The weak spots of proteins S3 and L1 have been variably visible. However, except for these two all the proteins of the complex have been highly reproducible.

The list of proteins bound to the immobilized tRNA is given in table 1.

The main differences between the simple sum of the separately-bound 30 S and 50 S subunit proteins and that found in the 70 S ribosomal protein-tRNA complex, are the following:

- (i) The absence of proteins L33 and L34 in the complex.
- (ii) The presence of protein S21 which was not

present in the complex when only 30 S subunit proteins were used [3].

The finding of protein S21 in the complex is not surprising because there is evidence demonstrating its proximity to the mRNA binding site [6], and the proteins S12 [7] and S18 [8], as well as its influence on the binding of aminoacylated tRNA to ribosomes [9]. The absence of some proteins and the appearance of the new one indicates that there is a cooperativity between 30 S and 50 S ribosomal subunit proteins in the binding to the immobilized tRNA.

Here, we want to stress that ultraviolet-induced crosslinking of ribosomal proteins to aa-tRNA within the ribosome gave qualitatively similar results [10].

A remarkable feature of the complex is that most of its proteins were also shown to be able to bind independently to rRNAs. This is particularly true of 30 S subunit proteins. However, we do not think that this ability of proteins puts the specificity of the tRNA-protein complex into question. After all, in view of the fact that almost all of 30 S subunit proteins seem to interact in a site-specific manner with 16 S RNA or with its large fragments (reviewed [11]), the interaction of certain proteins with both 16 S RNA and tRNA is simply unavoidable.

A good agreement of our data with the immuno-electron microscopic mapping of proteins on the 30 S ribosomal subunit models is obvious (fig.2A,B).

According to the model [12] (fig.2A), IgG binding sites for all 30 S subunit proteins but S6, involved in the immobilized tRNA-protein complex (S4, S7, S9, S12, S13, S18, S20, S21) are located close to each other on the smaller head of the 30 S subunit, forming in this way a well-localized domain. On the model [14] (fig.2B) all the proteins of the complex but S12,

Table 1
E. coli 70 S ribosomal proteins identified in the complex with immobilised tRNA at various MgCl₂ concentrations

	MgCl ₂ 1 mM, KCl 200 mM ^a	MgCl ₂ 30 mM, KCl 100 mM ^a
Small subunit	S4, S6, S7, S9, S12, S13, S18, S20, S21	S4, S6, S7, S9, S12, S13, S18, S20 (=L26)
Large subunit	L2, L6, L15, L16, L17, L18, L22, L26	L2, L16, L17, L20, L22, L26 (=S20)

^a Other buffer components are indicated in the text

